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RPC53 Encodes a Subunit of *Saccharomyces cerevisiae* RNA Polymerase C (III) Whose Inactivation Leads to a Predominantly G_1 Arrest

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***RPC53* is shown to be an essential gene encoding the C53 subunit specifically associated with yeast RNA polymerase C (III). Temperature-sensitive *rpc53* mutants were generated and showed a rapid inhibition of tRNA synthesis after transfer to the restrictive temperature. Unexpectedly, the *rpc53* mutants preferentially arrested their cell division in the G_1 phase as large, round, unbudded cells. The *RPC53* DNA sequence is predicted to code for a hydrophilic M_r-46,916 protein enriched in charged amino acid residues. The carboxy-terminal 136 amino acids of C53 are significantly similar (25% identical amino acid residues) to the same region of the human BN51 protein. The BN51 cDNA was originally isolated by its ability to complement a temperature-sensitive hamster cell mutant that undergoes a G_1 cell division arrest, as is true for the *rpc53* mutants.**

The eukaryotic RNA polymerases are complex enzymes composed of multiple, distinct subunits. *Saccharomyces cerevisiae* RNA polymerase C activity is associated with a complex of at least 13 different polypeptides ranging from 10 to 160 kDa (4, 11, 13, 49, 56). A subset of the yeast RNA polymerase C subunits is homologous to the eubacterial RNA polymerase core enzyme. C160 and C128 are homologous to the *Escherichia coli* β and β′ subunits, respectively (1, 24). Two molecules of the α subunit are found in *E. coli* RNA polymerase. AC40 and AC19 each have a domain similar to a functionally important domain of the α subunit (8). It was thus proposed that one copy each of AC40 and AC19 in RNA polymerases A and C is functionally homologous to the α homodimer of the *E. coli* RNA polymerase. A homodimer of the B44.5 subunit is likely to represent the α homolog of the B enzyme (29, 30). The two largest subunits along with the α homologs probably perform many of the same functions in polymerase assembly and the basic catalysis of transcription as do their homologs in the well-studied eubacterial enzyme. In contrast to these four core subunits, a function has not yet been assigned to the remaining nine small subunits. Five of these subunits (ABC27, ABC23, ABC14.5, ABC10α, and ABC10β) are shared between all three nuclear polymerases and thus probably contribute to a common eukaryotic (and possibly archaebacterial) core enzyme (4, 56). The four small subunits specifically associated with RNA polymerase C (C82, C53, C34, and C31) seem destined to perform functions specific to transcription by this polymerase. The C82 (6), C34 (52), and C31 (37) proteins have all been shown to be necessary for yeast cell viability and for the synthesis of tRNA by RNA polymerase C. In this report, we show that C53 also performs an essential cellular function required for tRNA synthesis. Furthermore, we report a sequence similarity between C53 and the BN51 protein that may encode the human homolog of C53.

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MATERIALS AND METHODS

Strains and media. The yeast strains used in this study are described in Table 1. CMY356 is a haploid meiotic segregant obtained after sporulation of CMY242 transformed with the plasmid pEMBLYc32-*RPC53*. CMY396 was derived by growing log-phase CMY356 cells in yeast extract-peptone-dextrose (2%) (YPD) in the presence of 4 μg of acriflavine per ml over three generations. A [*rho*^{0}] segregant was identified by its inability to use glycerol as a carbon source and by the absence of mitochondrial DNA as determined by fluorescent staining with 4′,6′-diamidino-2-phenylindole (DAPI) (see Fig. 7E). CMY397 is a mitotic segregant of CMY396 in which pEMBLYc32-*RPC53* was lost.

Yeasts were cultivated in YPD or in synthetic complete medium (50).

DNA sequencing of *RPC53*. The sequence (see Fig. 2) of the 2,414-bp *RPC53* *Hin*dIII-*Nru*I fragment (Fig. 1C) was determined by first subcloning portions of the fragment in either M13 or Bluescript (Stratagene) vectors in both orientations. The DNA sequence of these fragments was determined by the dideoxynucleotide method with a modified T7 DNA polymerase (Sequenase; United States Biochemical Corp.). As described in the Results, we unexpectedly observed that the 2.4-kb *RPC53* *Hin*dIII-*Nru*I fragment cloned from the AB320 yeast strain (38) could complement an *rpc53*::*HIS3-2* lethal disruption when cloned in the pUN20 (*TRP1 SUP11 ARS1 CEN4*) vector (10), but not when cloned in YCp50 (*URA3 ARS1 CEN4*) (46). We eventually ascribed this result to an inhibitory plasmid context effect of YCp50 on the expression of *RPC53*. However, since pUN20 carries a suppressor tRNA gene, whereas YCp50 does not, we initially wanted to exclude the possibility that the *RPC53* gene obtained from the AB320 strain contained a nonsense mutation. We thus cloned directly the corresponding 2.4-kb *Hin*dIII-*Nru*I *RPC53* fragment from strain LL20 (39) in the vector pEMBLYc32 (37). The *RPC53* DNA sequences from strains LL20 and AB320 were both determined. Shown in Fig. 2 is the DNA sequence found in strain LL20. The sequence of the *RPC53* gene in strain AB320 did not contain any nonsense mutations, but did show a number of sequence

TABLE 1. Yeast strains used

Strain	Genotype
CMY214	a/α *trp1 Δ1/trp1 Δ1 ura3-52/ura3-52 his3Δ200/his3Δ200 lys2-801/lys2-801 ade2-101/ade2-101 can1/+*
CMY242	CMY214 *rpc53::HIS3-1/+*
CMY274	a *trp1Δ1 his3Δ200 lys2-801 ade2-101 can1 RPC53*::pEMBLYi32(*URA3*)
CMY292	a *trp1Δ1 his3 Δ200 lys2-801 ade2-101 can1 rpc53::HIS3-1*/pUN20-*RPC53*
CMY356	a *trp1Δ1 his3 Δ200 lys2-801 ade2-101 can1 rpc53::HIS3-1*/pEMBLYc32-*RPC53*
CMY370	CMY214 *rpc53::HIS3-2/+*
CMY381	a *trp1Δ1 his3 Δ200 lys2-801 ade2-101 can1 rpc53::HIS3-2*/pEMBLYc32-*rpc53-ts28*
CMY383	a *trp1Δ1 his3 Δ200 lys2-801 ade2-101 can1 rpc53::HIS3-2*/pUN20-*RPC53*
CMY384	a *trp1Δ1 his3 Δ200 lys2-801 ade2-101 can1 rpc53::HIS3-2*/pEMBLYc32-*RPC53*
CMY323[a]	(RY262) α *rpb1-1 ura3-52*
CMY388[b]	α *cdc9 his7 leu2 can1 ura3 hom3 sap3*
CMY396	a *trp1Δ1 his3Δ200 lys2-801 ade2-101 can1 rpc53::HIS3-1* [*rho*^{0}]/pEMBLYc32-*RPC53*
CMY397	a *trp1Δ1 his3Δ200 lys2-801 ade2-101 can1 rpc53::HIS3-1* [*rho*^{0}]
SC12	a *leu2Δ1 trp1Δ1 his3Δ200 lys2-801 ade2-101 can1 rpc53::HIS3-2*/pEMBLYc32-*RPC53*

[a] A gift from Rick Young.
[b] A gift from Lee Hartwell.

differences relative to the *RPC53* sequence found in LL20. Five substitutions were found in third codon positions having no effect on the predicted C53 amino acid sequence: C303T, A570G, C633T, A885G, and A984G (the numbering scheme refers to that shown in Fig. 2). Two substitutions were found leading to conservative amino acid changes in the predicted C53 proteins: G417C leading to the substitution E139D, and C833T leading to the substitution A276V. Two more consequential sequence differences were found: a deletion of 6 bp, 793 to 798, leading to a deletion of two amino acids (GlyLeu, 255 and 256), and an inversion of 4 bp, 858 to 861, leading to a change of two residues, from LysArg (286 and 287) to AsnAla. All these variations represent sequence polymorphisms without effect on the function of the C53 protein as the *RPC53* genes from LL20 and AB320 are equally capable of complementing the *rpc53::HIS3-2* mutation when cloned in pUN20 or pEMBLYc32 vectors.

Physical mapping of *RPC53*. We initially mapped the *RPC53* gene to the left arm of chromosome 4 by the chromosome fragmentation method (62). An *RPC53* fragment was cloned in both orientations in the ARS fragmentation vector YCF3 and the CEN fragmentation vector YCF4. After linearization of the plasmids at the junction between the *RPC53* and vector sequences, stable transformants of a diploid yeast strain were obtained. Orthogonal-field-alternation gel electrophoresis (OFAGE) of chromosomes from transformants showed that in one orientation, the ARS fragmentation vector transformants carried a chromosome 4 derivative with a 200- to 250-kb deletion, whereas in the opposite orientation, the CEN vector transformants displayed a chromosome fragment of 200 to 250 kb. Southern blotting analysis of this gel showed that the *HO* gene hybridized to the 200- to 250-kb chromosome fragment. These results indicated that the *RPC53* gene was about 200 to 250 kb from the left end of chromosome 4. After sequencing the *RPC53* gene, we noticed the presence of a rare *Not*I sequence within its coding region. When the *Not*I-*Sfi*I map of the *S. cerevisiae* genome became available (32), we attempted to identify the *RPC53 Not*I site. There are three *Not*I sites within the first 250 kb of the left arm of chromosome 4. We probed a membrane containing yeast chromosomal DNA separately digested with *Not*I or *Sfi*I and separated by orthogonal-field-alternation gel electrophoresis to localize the *RPC53 Not*I site (see Fig. 4A). This blot was a generous gift from Phil James, who digested yeast chromosomal DNA in situ in blocks of agarose (24). The hybridization pattern obtained after probing with an *RPC53 Pvu*II-*Xba*I 1-kb fragment that spanned the *Not*I site conclusively identified the *RPC53 Not*I site as the third of six such sites from the left end of the chromosome. This places the *RPC53* gene at 210 kb from the chromosome 4 left telomere, in good agreement with our estimation from the chromosome fragmentation method.

Genetic mapping of *RPC53*. With the knowledge that

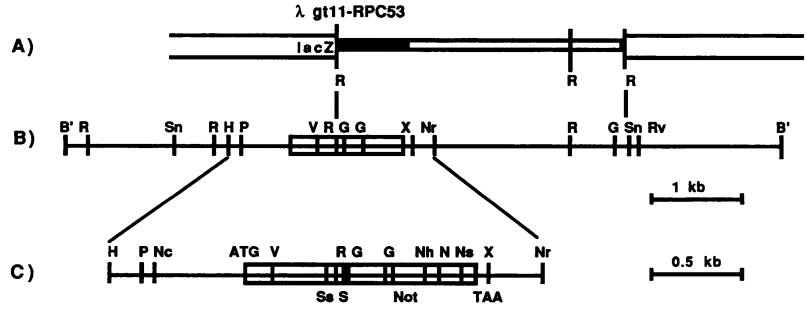


FIG. 1. Restriction maps of cloned *RPC53* fragments. (A) λgt11-*RPC53* clone obtained by immunoscreening with anti-C53 immunoglobulins (45). The solid portion represents *RPC53* coding sequences fused to the carboxy terminus of β-galactosidase. (B) Genomic fragment containing the complete *RPC53* gene obtained by screening a YRp7-*Sau*3A yeast genomic DNA bank (38) with the *Eco*RI DNA insert of λgt11-*RPC53*. (C) The 2,414-bp *RPC53 Hin*dIII-*Nru*I fragment whose DNA sequence was determined (see Fig. 2). B', *Bam*HI-*Sau*3A junction; G, *Bgl*II; H, *Hin*dIII; N, *Nae*I; Nc, *Nco*I; Nh, *Nhe*I; Ns, *Nsi*I; P, *Pst*I; R, *Eco*RI; Rv, *Eco*RV; S, *Sal*I; Sn, *Sna*BI; Ss, *Sst*I; V, *Pvu*II; X, *Xba*I.

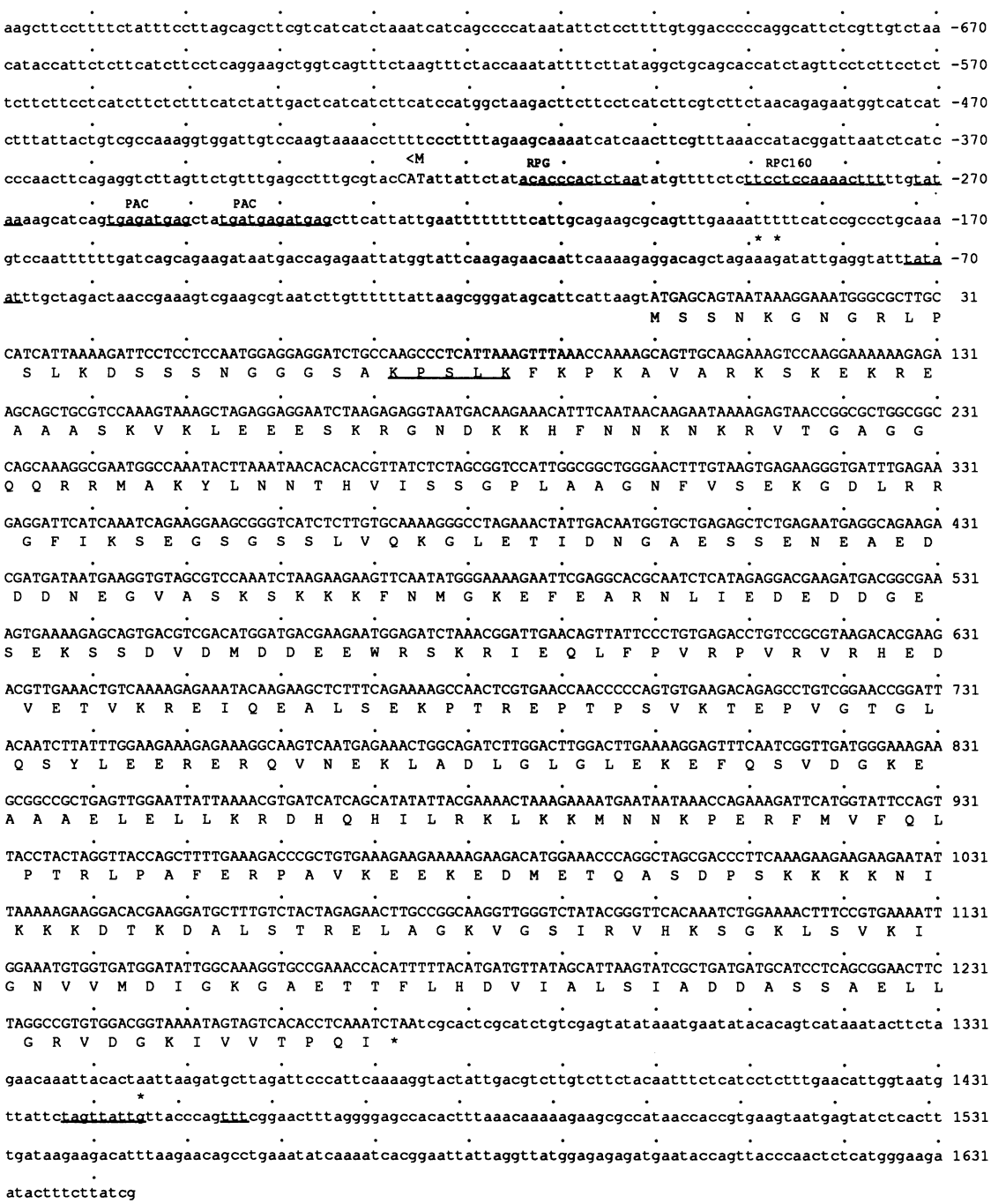


FIG. 2. Sequence of the 2,414-bp *RPC53 Hin*dIII-*Nru*I (Fig. 1C) DNA fragment and the predicted C53 amino acid sequence. This sequence has been assigned the EMBL Data Library accession number X63501. The asterisks at −87 and −89 are the major sites of transcription initiation, and the asterisk at 1446 is the major site of transcription termination as determined by S1 nuclease mapping (35). The underlined sequence KPSLK (amino acids 25 to 29) represents a possible nuclear localization signal (16). An RPG box [consensus sequence R(C/A)AYCCRYNCAYY (61)] at −314, two PAC boxes [consensus sequence TG(A/C)GATGAG (8)] at −258 and −246, a 16-bp sequence (−290) similar to a sequence found at −45 of the *RPC160* promoter (1), and two TATAA sequences at −272 and −73 were found in the *RPC53* promoter region. These sequences may contribute to transcriptional regulation of the *RPC53* gene. Note that a divergently oriented open reading frame encoding more than 148 amino acids was observed starting at −325 (indicated by <M above the initiating codon in the figure). The predicted protein sequence of this open reading frame is composed of 50% acidic amino acid residues.

RPC53 was about 200 kb from the left end of chromosome 4, we sought genetic linkage with other mutations in this region. The *RPC53* chromosomal locus was marked with *URA3* by targeted integration of a pEMBLYi32-*RPC53* plasmid. An 800-bp *Eco*RI-*Xba*I *RPC53* fragment was cloned into pEMBLYi32 (2), and the resulting plasmid was then cut within *RPC53* sequences with *Bgl*II to target its integration to the homologous site during lithium acetate transformation (20)

of CMY215. The integration of pEMBLYi32(*URA3*) to the *RPC53* locus was confirmed by Southern blot analysis for the transformant CMY274 used in the meiotic mapping experiments. CMY274 (*RPC53*::*URA3*) was crossed to *rpb1* (CMY323) and *cdc9* (CMY388) temperature-sensitive mutant strains. After sporulation of the resulting diploid strains, tetrad analysis showed *RPC53*::*URA3* to lie 1.5 centimorgans from *rpb1* and 4 centimorgans from *cdc9* (see Fig. 4B).

Disruption of *RPC53*. A pBR322-*rpc53*::*HIS3-1* plasmid was created by first subcloning the 2.4-kb *Hin*dIII-*Nru*I *RPC53* fragment in pBR322 and then substituting the internal 207-bp *Bgl*II *RPC53* fragment with the 1.7-kb *Bam*HI *HIS3* fragment (54). Digestion of this plasmid with *Nco*I and *Nru*I liberates a 3.6-kb *rpc53*::*HIS3-1* fragment (see Fig. 5) that was used for gene disruption (47) of one copy of the *RPC53* gene in the diploid yeast strain CMY214.

An essentially complete deletion of the *RPC53* gene was made by first subcloning the 2.4-kb *Hin*dIII-*Nru*I *RPC53* fragment in YCp50 (46). After digestion of this plasmid with *Pvu*II, a short treatment with nuclease *Bal* 31 was performed to delete *RPC53* coding sequences. This was followed by digestion with *Xba*I, the filling-in of protruding ends with the Klenow DNA polymerase, and recircularization of the plasmid with T4 DNA ligase in the presence of *Bam*HI linker oligonucleotides. After recovery in *E. coli*, a plasmid was found containing a deletion of all but approximately 60 bp of *RPC53* coding sequence with a *Bam*HI linker at the site of the deletion. The size of the deletion was estimated by the gel migration of restriction fragments. The 1.7-kb *Bam*HI *HIS3* fragment was then inserted into this plasmid to yield a YCp50-*rpc53*::*HIS3-2* construction (see Fig. 5). Digestion of this plasmid with *Nco*I and *Nru*I liberates a 2.6-kb *rpc53*::*HIS3-2* fragment that was used to delete one of the chromosomal *RPC53* genes in the CMY214 diploid yeast strain.

In vitro mutagenesis of *RPC53*. The pEMBLYc32-*RPC53* plasmid was converted to single-stranded form by superinfecting *E. coli* JM107 cells containing this plasmid with the R408 F1 helper bacteriophage (48). A gapped heteroduplex (42) was then formed after denaturation of double-stranded pEMBLYc32 vector (digested at the sites used for subcloning the *RPC53* fragment) in the presence of single-stranded pEMBLYc32-*RPC53* DNA; this was followed by a period of renaturation. The resulting heteroduplex molecules are composed of double-stranded vector sequences with only the *RPC53* fragment remaining single stranded. Treatment of these heteroduplexes with sodium bisulfite specifically restricts the GC-to-AT transition mutations to the *RPC53* fragment. Gapped heteroduplexes were incubated with 4 M sodium bisulfite at 37°C for 10, 20, or 40 min, and the reactions were stopped and the DNA purified as previously described (33). The mutagenized heteroduplexes were then used to transform directly the *E. coli ung* strain BD1528 (42) to repair the single-stranded gap and to fix the C-to-U transition mutations. Conditional *rpc53* mutants were then screened for by a plasmid-shuffle test. Mutagenized DNA banks were used to transform the yeast strain CMY383 *rpc53*::*HIS3-2*/pUN20-*RPC53*, and conditional mutants (cold and heat sensitive) were sought for as transformants unable to grow at the restrictive temperature after counterselecting the pUN20(*SUP11*)-*RPC53* plasmid by replica plating transformants to YPD plus 2.5 M ethylene glycol plates (10). Mutations were shown to be plasmid linked by recovering the pEMBLYc32-*RPC53* in *E. coli* and showing that the same conditional mutant phenotype was uniformly obtained after retransformation of the CMY383 tester strain with the purified mutant plasmid.

Continuous radioactive labeling of protein and RNA. CMY397 *rpc53*::*HIS3-1* [*rho*^{0}] and the isogenic wild-type CMY396 *rpc53*::*HIS3-1* [*rho*^{0}]/pEMBLYc32-*RPC53* cells were grown at 23°C in synthetic complete medium containing 10 μg of uracil per ml to an optical density at 660 nm of 0.1. [^{3}H]uracil (50 Ci/mmol) and [^{35}S]methionine (500 Ci/mmol) were then added to the cultures to a concentration of 1 and 0.5 μCi/ml, respectively. The cultures were transferred to 38°C 1 h after addition of the radioactive precursors. Incorporation of radioactivity into macromolecules (see Fig. 6D) was followed by trichloroacetic acid precipitation: 4 ml of cold 5% trichloroacetic acid was added to 1 ml of culture, and the precipitate was collected by filtration onto glass fiber filters and then extensive washing with 5% trichloroacetic acid. The filters were then washed with ethanol, dried, and placed in liquid scintillation fluid for the simultaneous counting of tritium and ^{35}S.

The increase in cell number and optical density as well as the percentage of unbudded cells were monitored in parallel unlabeled cultures (see Fig. 6A, B, and C).

Labeling and analysis of small, stable RNAs. CMY396, CMY397, CMY381, and CMY384 cells were grown at 23°C in synthetic complete medium containing 5 μg of uracil per ml to an optical density (660 nm) of 0.4. For each labeling, 20 ml of cells was then transferred to either 37 or 38°C for 30 min, 3 h, or 6 h (the temperature and time of incubation for the different strains are as indicated in Fig. 9). RNA was labeled by adding to each culture [^{3}H]uracil (50 Ci/mmol) to a concentration of 15 μCi/ml for 1 h. Labeled cells were centrifuged and resuspended in 5 ml of extraction buffer (100 mM Tris-HCl [pH 7.5], 250 mM NaCl, 5 mM EDTA, 0.5% sodium dodecyl sulfate [SDS]) followed immediately by the addition of an equal volume of phenol. Small RNAs were extracted by vigorous agitation of this emulsion at 65°C for 1 h. RNA in the aqueous phase was then precipitated by the addition of 2 volumes of ethanol. The RNA pellet was next resolubilized in 0.1 ml of diethyl pyrocarbonate-treated water, and the radioactivity incorporated was determined and the yield of RNA was estimated by the sample A_{260} (an A_{260} of 1 was taken to represent 40 μg of RNA per ml). RNA (20 μg) for each sample was denatured in 90% formamide loading buffer before electrophoresis on a 6% polyacrylamide–7 M urea–1× TBE (Tris-borate-EDTA) gel to separate small RNAs. The gel was then briefly stained with 0.5 mg of ethidium bromide per ml to visualize the RNA and photographed before it was fixed in 7% acetic acid, treated with Amplify (Amersham) for fluorography, and dried. Fluorography was done at −70°C with preflashed Kodak XAR film.

Flow cytometry of yeast cells. For each sample to be analyzed, approximately 10^7 cells were fixed in 70% ethanol for at least 1 h. Cells were then washed once with 0.2 M Tris-HCl (pH 7.5) and resuspended in 0.1 ml of the same buffer containing 100 μg of RNase A. RNA was digested by incubating at 37°C for 2 h. Cells were then washed in phosphate-buffered saline (PBS) and resuspended in 0.1 ml of 50 μg of propidium iodide per ml in PBS for 20 min. After being washed with PBS, cells were resuspended in 5 μg of propidium iodide per ml in PBS at a concentration of about 5×10^6 cells per ml. Cells were lightly sonicated and observed by fluorescence microscopy to ensure specific nuclear staining before quantifying fluorescent emission with an OrthoDiagnostics Systems 2150 fluorescence-activated cell sorter (FACS).

Expression and functional testing of BN51 protein in yeast cells. Two galactose-inducible BN51 genes were constructed, one with the entire coding sequence and one with that portion of the sequence encoding the last 167 amino acids of the BN51 protein (starting from the internal methionine at position 228 of the amino acid sequence [22]). These BN51 sequences were placed under the control of the *GAL10* promoter of the Fusionator multicopy plasmid (a generous gift from Stephen Johnson, Department of Genetics, University of Washington). The Fusionator is a 2 μm-based plasmid containing the *LEU2* selectable marker (26). Oligonucleotides were used to amplify by polymerase chain reaction the BN51-coding sequences as *Sal*I-*Bam*HI DNA fragments starting from a human BN51 cDNA clone (19). The following primers were used for the amplification of the entire BN51-coding region: HBN3M = TTCCGTCGACCAT GTCGGAAGGAAACGC (5′ oligonucleotide) and HBN1V = GGGGGGATCCTTACCGGTGTTTGTGATCC (3′ oligonucleotide). A *Sal*I-*Bam*HI fragment encoding the last 167 BN51 amino acids was amplified by using the primers HBN2M (GGGGGTCGACCATGAAGGCTCCTCCCAAA GC [5′ oligonucleotide]) and the same 3′ oligonucleotide (HBN1V) as above. Cloning of these *Sal*I-*Bam*HI fragments into the same sites of the Fusionator polylinker placed the transcription of these coding sequences under the control of the *GAL10* promoter. Polymerase chain reaction-derived clones of these two plasmids were then introduced into the yeast strain SC12 by lithium acetate transformation (20). SC12 [*rpc53*::*HIS3-2*/pEMBLYc32(*URA3*)-*RPC53*] was used to test the ability of the BN51 proteins to complement a complete deletion of the *RPC53* gene. SC12 transformants expressing BN51 or BN51 carboxy-terminal sequences in a galactose-containing medium were tested for their ability to grow in the absence of the pEMBLYc32(*URA3*)-*RPC53* plasmid. Cells unable to lose this plasmid are sensitive to the drug 5-fluoro-orotic acid (3).

EMBL data library submission. The *RPC53* DNA sequence (Fig. 2) has been assigned the accession number X63501.

RESULTS

Cloning and sequencing of the *RPC53* gene. Two λgt11 clones potentially encoding a part of the *RPC53* gene were obtained by screening an expression library with antibodies prepared against the C53 subunit (45). Temperature-inducible lysogens of both clones produced a fusion protein about 30 kDa larger than β-galactosidase that reacted with both anti-C53 and anti-β-galactosidase antibodies. Extracts of bacteria producing this fusion protein were able to bind antibodies from the anti-C53 immunoglobulin preparation that were capable of specifically inhibiting RNA polymerase C transcription in vitro (35), thus indicating that we had likely isolated C53-coding sequences. Restriction enzyme analysis showed that the two clones contained a yeast DNA insert with a common *Eco*RI site ligated to the 3′ end of the *lacZ* gene of λgt11 (Fig. 1A). Since the fusion protein analysis indicated that only a portion of the *RPC53* coding sequence had been isolated, the complete gene was sought from a YRp7 yeast genomic DNA bank (38) by colony hybridization with the λgt11 yeast DNA insert. A 7.7-kb genomic DNA fragment was obtained that contained the complete gene (Fig. 1B). The DNA sequence of the 2,414-bp *Hin*dIII-*Nru*I fragment shown in Fig. 1C was determined. An open reading frame of 1,272 nucleotides corresponding to a protein of 424 amino acids (predicted M_r, 46916) was found

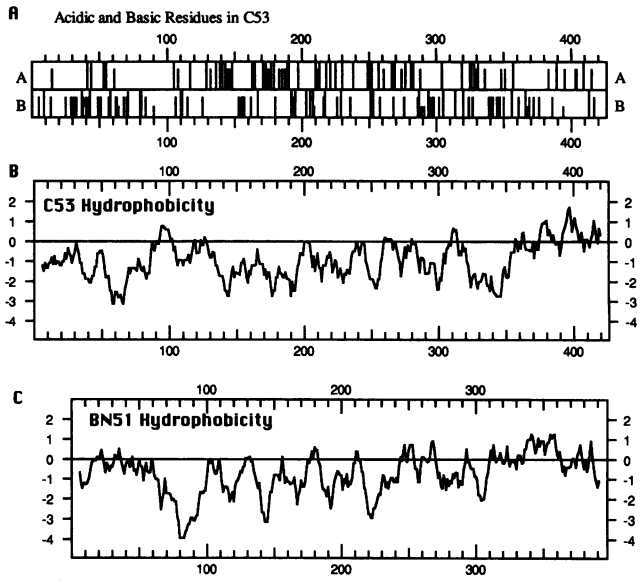


FIG. 3. (A) Graphic display of the positions of acidic (A) and basic (B) residues along the C53 protein sequence. The full bars in the A (acidic) row represent glutamate residues, and the lower bars represent aspartate residues. In the B (basic) row, the full bars represent arginine, the intermediate bars represent lysine, and the small bars represent histidine residues. (B and C) Kyte-Doolittle hydropathy plots for the C53 and BN51 proteins, respectively. Positive values represent hydrophobic regions, and negative values represent hydrophilic regions, around a window of 11 amino acids for each protein. Notice the overall similarity in the profiles for the two proteins. All the graphics for this figure were executed with the DNA Strider computer program (34).

(Fig. 2). This reading frame is the same as that fused to *lacZ* in the original λgt11 clone as determined by sequencing the fusion site of the phage DNA. The difference between the predicted molecular weight of C53 and that observed by SDS-polyacrylamide gel electrophoresis may be an inherent property of the sequence or may be due to posttranslational modifications in yeast cells. Both of these factors have been found to contribute to anomalous electrophoretic migration of other RNA polymerase subunits (56). No TACTAAC splicing signal (64) was found in the DNA sequence, so we assume that the gene does not contain an intron. Northern (RNA) blot and nuclease S1 protection analysis (35) indicated that *RPC53* is expressed as an mRNA of about 1,500 nucleotides with initiation and termination sites as shown in Fig. 2.

The deduced amino acid sequence of the C53 subunit is very hydrophilic throughout its length, excepting a carboxy-terminal 50-amino-acid region as shown in the hydropathy plot of Fig. 3B. This hydrophilicity is accounted for by a striking distribution of charged residues (Fig. 3A). An amino-terminal 125-amino-acid domain is highly basic and is followed by alternating clusters of acidic and basic residues. These structural features suggest that the C53 subunit lies on the surface of RNA polymerase C in an extended conformation. This suggestion is consistent with the extreme susceptibility of the C53 subunit to proteolytic degradation (19, 49) as well as the relative ease with which it may be dissociated from the polymerase (5, 63). C53 has a calculated isoelectric point of 9.2.

Sequence motifs potentially involved in nuclear transport

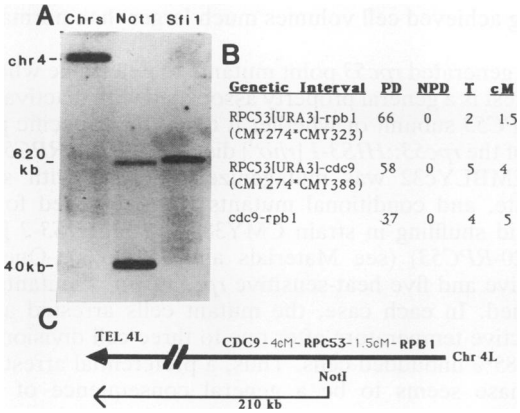


FIG. 4. Physical and genetic mapping of *RPC53*. (A) *S. cerevisiae* chromosomal DNA, before (Chrs lane) and after digestion with *Not*I and *Sfi*I (as indicated), was separated by pulsed-field gel electrophoresis, and the DNA was then transferred to a nylon membrane and probed with a radioactively labeled 1-kb *Pvu*II-*Xba*I *RPC53* fragment (Fig. 1C). This fragment contains sequences on either side of the *Not*I site found within the *RPC53* gene. The size of the hybridizing fragments identifies the *Not*I site as the third of six *Not*I sites from the left end of chromosome 4 (32). (B) Table summarizing the tetrad analysis indicating genetic linkage between a *URA3* plasmid integrated at the *RPC53* chromosomal locus and the *cdc9* and *rpb1* mutations. Shown are the number of tetrads in each case in which the two markers segregated as parental ditypes (PD), nonparental ditypes (NPD), or tetratypes (T). The genetic distance in centimorgans (cM) was calculated according to the equation cM = 50(T + 6 NPD)/(PD + NPD + T). (C) Diagram summarizing the physical and genetic mapping of the *RPC53* gene.

were observed in C53. The sequence KPSLK (amino acids 25 to 29) is of the form KPXXK, which serves as one class of nuclear targeting signal in *S. cerevisiae* (16). Some of the basic clusters in the protein also have sequences resembling other nuclear targeting signals (9, 12). These sequences may participate in transport of C53, with or without other associated RNA polymerase C subunits, into the nucleus.

Putative regulatory signals were also seen in sequences flanking the *RPC53* coding sequence (Fig. 2). The 5′ noncoding region contains two candidate TATA box sequences, a 16-bp sequence that is similar to a sequence found in the *RPC160* promoter, an RPG box sequence, and a tandem repetition of the PAC box. The RPG box is an upstream activating sequence for many genes (61), including those encoding proteins involved in protein and stable RNA synthesis. The RPG sequence is bound by the GRF1/RAP/TUF protein (61). The PAC box is a sequence found in the promoter region of all 11 genes examined to date encoding subunits specific to RNA polymerases A (I) or C (III) with the exception of *RPC160* (8).

Physical and genetic mapping of the *RPC53* gene. The *RPC53* gene was estimated to lie between 200 and 250 kb from the left telomere of chromosome 4 of *S. cerevisiae* by the chromosome fragmentation method of mapping (see Materials and Methods). The presence of a rare *Not*I site in the *RPC53* gene allowed a precise determination of its physical map position. *RPC53* DNA hybridized to *Not*I bands of 40 and 620 kb as well as to an *Sfi* band of 635 kb (Fig. 4A). This pattern identifies the *Not*I site of RPC53 as the third of six *Not*I sites from the left telomere of chromosome 4 in the Link and Olson *Not*I-*Sfi*I map of the yeast genome (32) and places it at 210 kb from the left end of the chromosome. Genetic mapping of a *URA3* insertion at the *RPC53* locus showed the *RPC53* locus to be tightly linked to *CDC9* and *RPB1* (Fig. 4B).

***RPC53* encodes an essential gene.** Two different disruptions of the *RPC53* gene were constructed to determine whether the gene is essential. In the *rpc53*::*HIS3-2* disruption, all but approximately 60 nucleotides of the *RPC53* coding sequence were substituted with a *HIS3* DNA fragment (Fig. 5A). A diploid strain heterozygous for the *rpc53*::*HIS3-2* substitution gave rise after sporulation to tetrads containing no more than two viable spores that were invariably auxotrophic for

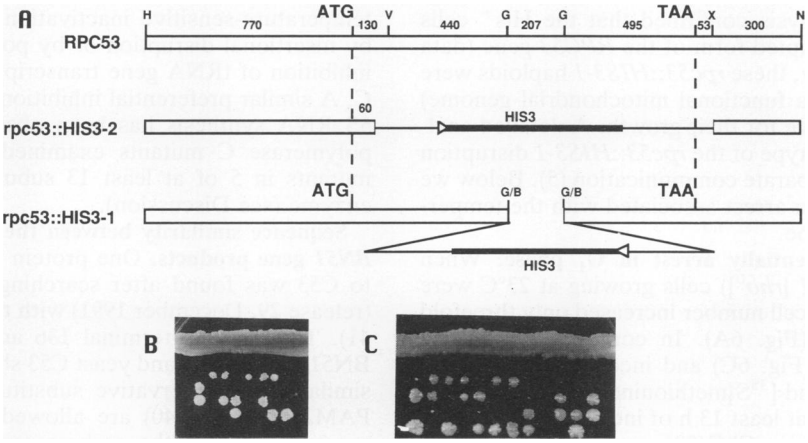


FIG. 5. Effect of disrupting the *RPC53* gene. (A) Shown are two different *RPC53* disruption constructions. The *rpc53*::*HIS3-2* disruption deletes all but some 60 bp of *RPC53* and substitutes the 1.7-kb *Bam*HI *HIS3* fragment (54). The *rpc53*::*HIS3-1* disruption replaces only the internal 207-bp *RPC53 Bgl*II fragment with the 1.7-kb *Bam*HI *HIS3* fragment. Restriction sites are as defined in the legend to Fig. 1. The orientation of the *HIS3* gene is indicated. (B) Tetrad dissection of asci obtained after sporulation of CMY370 (**a**/α *rpc53*::*HIS3-2*/+). No more than two viable spores were obtained, and these were always auxotrophic for histidine. The absence of viable *rpc53*::*HIS*$^+$ spore colonies suggests that deletion of *RPC53* creates a recessive lethal mutation. (C) Tetrad dissection of asci obtained by sporulation of CMY242 (**a**/α *rpc53*::*HIS3-1*/+). Ten to fifteen percent of the spores gave rise to colonies growing considerably more slowly than their sister-spore colonies after 10 days of growth at 23°C. The slow-growing cells always proved to be of genotype *rpc53*::*HIS3-1* [*rho*$^-$] and were temperature sensitive for their growth.

histidine (Fig. 5B). Microscopic examination of the *rpc53*::*HIS3-2* spores showed that most had germinated and undergone one cell division before arresting as unbudded cells. The absence of His^+ haploid spores suggested that the *RPC53* deletion is lethal. This possibility was tested by cloning the *Hin*dIII-*Nru*I fragment containing the *RPC53* gene (Fig. 1C) into the centromere vectors pUN20 (*TRP1 SUP11*) and YCp50 (*URA3*) and then separately introducing each plasmid into the *rpc53*::*HIS3-2*/+ diploid strain. After sporulation, haploid *rpc53*::*HIS3-2*/pUN20-*RPC53* segregants were readily obtained. These cells were incapable of losing the *RPC53* plasmid as determined by a plasmid-sectoring assay based on colony color as well as by their inability to grow on YPD containing 2.5 M ethylene glycol, which kills cells carrying the *SUP11* gene on pUN20 (10). Thus, *RPC53* is indeed an essential gene. Unexpectedly, the same *RPC53* fragment that complemented the *rpc53*::*HIS3-2* substitution when present on pUN20 was unable to do so when present on YCp50. To investigate this further, we transferred the *Hin*dIII-*Nru*I *RPC53* fragment from YCp50 to the centromeric vector pEMBLYc32(*URA3*) (37). The YCp50-*RPC53* and pEMBLYc32-*RPC53* plasmids were introduced into strain CMY383 (*rpc53*::*HIS3-2*/pUN20-*RPC53*), and transformants were tested for their ability to lose the resident pUN20-*RPC53*. By this plasmid-shuffle test, pEMBLYc32-*RPC53* was capable of complementing the *rpc53*::*HIS3-2* mutation, whereas YCp50-*RPC53* could not. The 2.4-kb *Hin*dIII-*Nru*I fragment, containing 760 bp upstream and 360 bp downstream of the *RPC53* coding sequence, appears to contain all the promoter and termination signals necessary for *RPC53* expression (Fig. 2). Thus, we believe that *RPC53* expression must be inhibited in an unknown manner when presented in the context in which it was inserted in YCp50. Plasmid context effects on gene expression have also been observed for the yeast *TRP1* and *HIS3* genes (53).

A disruption of the *RPC53* gene was also constructed by replacing a 207-bp *Bgl*II fragment encoding amino acids 193 to 259 with *HIS3* DNA. Sporulation of diploids heterozygous for this *rpc53*::*HIS3-1* disruption occasionally gave rise to slow-growing His^+ colonies after germination at 23°C (Fig. 5C). Southern blot analysis confirmed that the His^+ cells contained only the disrupted form of the *RPC53* gene (data not shown). Surprisingly, these *rpc53*::*HIS3-1* haploids were always [*rho*$^-$] (lacking a functional mitochondrial genome) and temperature sensitive for their growth. A detailed analysis of the [*rho*$^-$] phenotype of the *rpc53*::*HIS3-1* disruption will be described in a separate communication (5). Below we describe the cell division arrest associated with the temperature-sensitive phenotype.

***rpc53* mutants preferentially arrest in G_1 phase.** When CMY397 (*rpc53*::*HIS3-1* [*rho*^{0}]) cells growing at 23°C were transferred to 38°C, the cell number increased only threefold before division arrest (Fig. 6A). In contrast, the optical density of the culture (Fig. 6C) and incorporation of [^{3}H]uracil into total RNA and [^{35}S]methionine into total protein increased steadily over at least 13 h of incubation (Fig. 6D). A congenic control strain, CMY396 *rpc53*::*HIS3-1* [*rho*^{0}]/pEMBLYc32-*RPC53*, grew and divided continuously at 38°C. Most of the mutant cells arrested their division as large, unbudded cells with a single nucleus (Fig. 6B and 7). The morphology of the cells suggested that they were arrested in the G_1 phase of the cell cycle, and analysis by flow cytometry confirmed that most cells had a G_1 content of DNA (Fig. 8). The mutant cells retained good viability (85%) over at least 12 h at the restrictive temperature despite their having achieved cell volumes much larger than normal (Fig. 7).

We generated *rpc53* point mutants to determine whether a G_1 arrest is a general property associated with inactivation of the *RPC53* subunit or whether it might be a specific phenotype of the *rpc53*::*HIS3-1* [*rho*^{0}] disruption. The *RPC53* gene in pEMBLYc32 was mutagenized in vitro with sodium bisulfite, and conditional mutants were screened for after plasmid shuffling in strain CMY383 (*rpc53*::*HIS3-2* [*rho*$^+$]/pUN20-*RPC53*) (see Materials and Methods). One cold-sensitive and five heat-sensitive *rpc53* [*rho*$^+$] mutants were obtained. In each case, the mutant cells arrested at their restrictive temperature after one to three cell divisions with 65 to 85% unbudded cells. Thus, a preferential arrest in the G_1 phase seems to be a general consequence of Rpc53 inhibition.

Synthesis of tRNA is inhibited in *rpc53* mutants. Incorporation of [^{3}H]uracil into total RNA (Fig. 6D) was not greatly affected after transfer of the temperature-sensitive strain CMY397 (*rpc53*::*HIS3-1* [*rho*^{0}]) to 38°C. As most of this synthesis is represented by the large rRNAs under these continuous labeling conditions, it can be concluded that RNA polymerase A (I) is functional in the *rpc53*::*HIS3-1* [*rho*^{0}] strain. The C53 protein is specifically associated with RNA polymerase C, and anti-C53 antibodies inhibit the in vitro transcription of a tRNA gene (19). We thus examined whether RNA polymerase C transcription was affected in *rpc53* mutants by labeling RNA for 60 min with [^{3}H]uracil and then extracting and electrophoretically separating the small RNAs on polyacrylamide gels. Transfer RNA synthesis was severely inhibited in CMY397 (*rpc53*::*HIS3-1* [*rho*^{0}]) cells after 6 h of incubation at 38°C in comparison with the CMY396 (*RPC53*$^+$ [*rho*^{0}]) congenic control strain (Fig. 9A and B). In contrast, 5S RNA, also transcribed by RNA polymerase C, and the polymerase A-derived 5.8S RNA were little or not affected in the *rpc53*::*HIS3-1* strain over this period.

We also examined the synthesis of small RNAs in the strain CMY381 (*rpc53*::*HIS3-2* [*rho*$^+$])/pEMBLYc32-*rpc53-ts*28. A specific inhibition of tRNA synthesis was seen in this strain within 30 min of transfer to 37°C (Fig. 9C). Thus, temperature-sensitive inactivation of *RPC53* caused either by insertional disruption or by point mutations leads to an inhibition of tRNA gene transcription by RNA polymerase C. A similar preferential inhibition of tRNA compared with 5S RNA synthesis has been observed for all yeast RNA polymerase C mutants examined to date. These include mutants in 5 of at least 13 subunits that compose the C enzyme (see Discussion).

Sequence similarity between the yeast *RPC53* and human *BN51* gene products. One protein with significant similarity to C53 was found after searching the EMBL data library (release 29, December 1991) with the TFASTA program (40, 41). The carboxy-terminal 136 amino acids of the human BN51 protein (22) and yeast C53 share 25% identity and 78% similarity if conservative substitutions as dictated by the PAM250 matrix (40) are allowed. The FASTA similarity score of 166 for the carboxy-terminal region of the two proteins is statistically significant; the match is 8 standard deviations above the mean for a comparison between C53 and shuffled BN51 sequences by the RDF2 program (40, 41). Weaker similarities are seen for the amino-terminal regions of the two proteins (Fig. 10). Furthermore, the overall size (395 amino acids for BN51, 424 amino acids for C53) and overall hydrophilicity (compare Fig. 3B and C) of the two proteins are similar. A direct test of functional homology

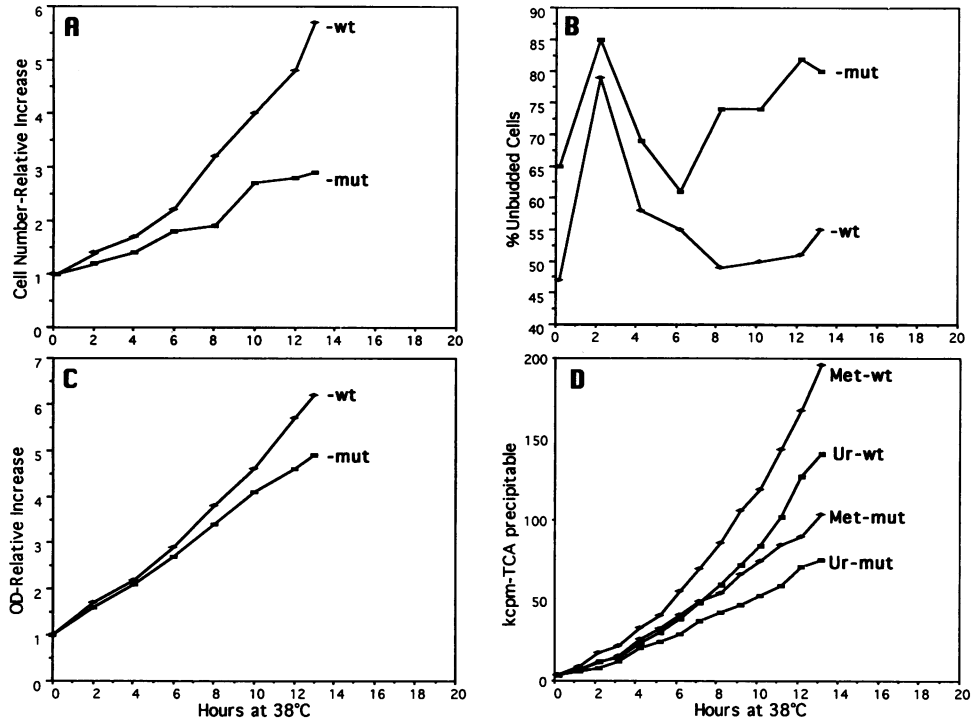


FIG. 6. Characterization of the temperature-sensitive phenotype associated with the *rpc53::HIS3-1* disruption mutation. CMY397 *rpc53::HIS3-1* [*rho*^{0}] (designated mut in the figure) and isogenic CMY396 *rpc53::HIS3-1* [*rho*^{0}]/pEMBLYc32-*RPC53* (designated wt) cells were grown in synthetic complete medium at 23°C to the early log phase before being transferred to 38°C for the times indicated in the figure. (A) Relative increase in cell number. Cultures of wild-type and mutant cells were shifted to 38°C at an initial value of 2×10^6 cells per ml. (B) Percentage of unbudded cells. The initial accumulation of unbudded cells within the first few hours of transfer to 38°C is due to a transient heat shock inhibition of the cell cycle in the G_1 phase (28). Note that both wild-type and mutant cells recovered from the heat shock effect but that only the mutant cells rearrested as unbudded cells at the end of the next division. (C) Relative increase in the optical density (OD) of cultures. Mutant and wild-type cell cultures were transferred to 38°C at an A_{660} of 0.15. (D) Continuous labeling of total cellular protein and RNA. [^{35}S]methionine and [^{3}H]uracil were added to cell cultures 1 h before transfer to 38°C. Incorporation of radioactive precursors was followed by trichloroacetic acid (TCA) precipitation. Note that mutant cells continued to accumulate protein and RNA during the arrest period.

between the two proteins was attempted by placing BN51 human cDNAs under the control of the yeast *GAL10* promoter on a multicopy plasmid (see Materials and Methods). No complementation of the lethal *rpc53::HIS3-2* disruption was observed when either the entire BN51-coding sequence or that portion encoding the last 167 amino acids of the protein (the region exhibiting the most similarity with C53 and corresponding to the functionally essential portion of C53 [5]) were expressed from the *GAL* promoter. However, preliminary results have indicated that the BN51 protein does not accumulate in these yeast strains, and further work will be necessary before a definitive conclusion can be drawn regarding the capacity of BN51 to functionally replace C53.

DISCUSSION

***RPC53* encodes an RNA polymerase C-specific subunit required for tRNA gene transcription.** A role for C53 in RNA polymerase C function has previously been found in experiments in which anti-C53 antibodies were seen to inhibit tRNA gene transcription in vitro (19). In this report, we show that a functional C53 protein is required for yeast cell viability and that inactivation of C53 temperature-sensitive mutants rapidly leads to an inhibition of tRNA gene transcription in vivo. The rapidity of the inhibition in the *rpc53::HIS3-1* and *rpc53-ts*28 mutants (Fig. 9) indicates that these C53 mutant proteins are temperature sensitive for their function or their stability after they associate with the other RNA polymerase C subunits. Mutants that are uniquely temperature sensitive for the assembly of RNA polymerase C, such as the *rpc40-ts* mutants (33), exhibit decreased levels of transcription only after several generations of growth at the restrictive temperature. The genetic data are thus consistent with the antibody inhibition studies in suggesting a direct role for the C53 subunit in tRNA synthesis. Analysis of in vitro transcription by RNA polymerase C purified from C53 mutants should permit a more precise delineation of its role in the transcriptional process (5).

A preferential inhibition of tRNA compared with 5S RNA synthesis in vivo is a property of all RNA polymerase C mutants examined to date. Aside from C53 (Fig. 9), this includes mutants of the C160 (15), C82 (6), C34 (52), and C31 (37) subunits. In the C31 subunit, a nonsense mutation weakly suppressed by a tRNA inserting the homologous amino acid led to a specific inhibition of tRNA synthesis. Thus, a simple reduction in the amount of the wild-type RNA polymerase C preferentially affects tRNA compared with 5S RNA synthesis. On the other hand, at least a part of the *rpc160* and *rpc53* mutant phenotypes was due to an enhanced thermolability of the mutant polymerase. There are at least two likely possibilities to explain the heightened resistance of 5S RNA synthesis compared with tRNA syn-

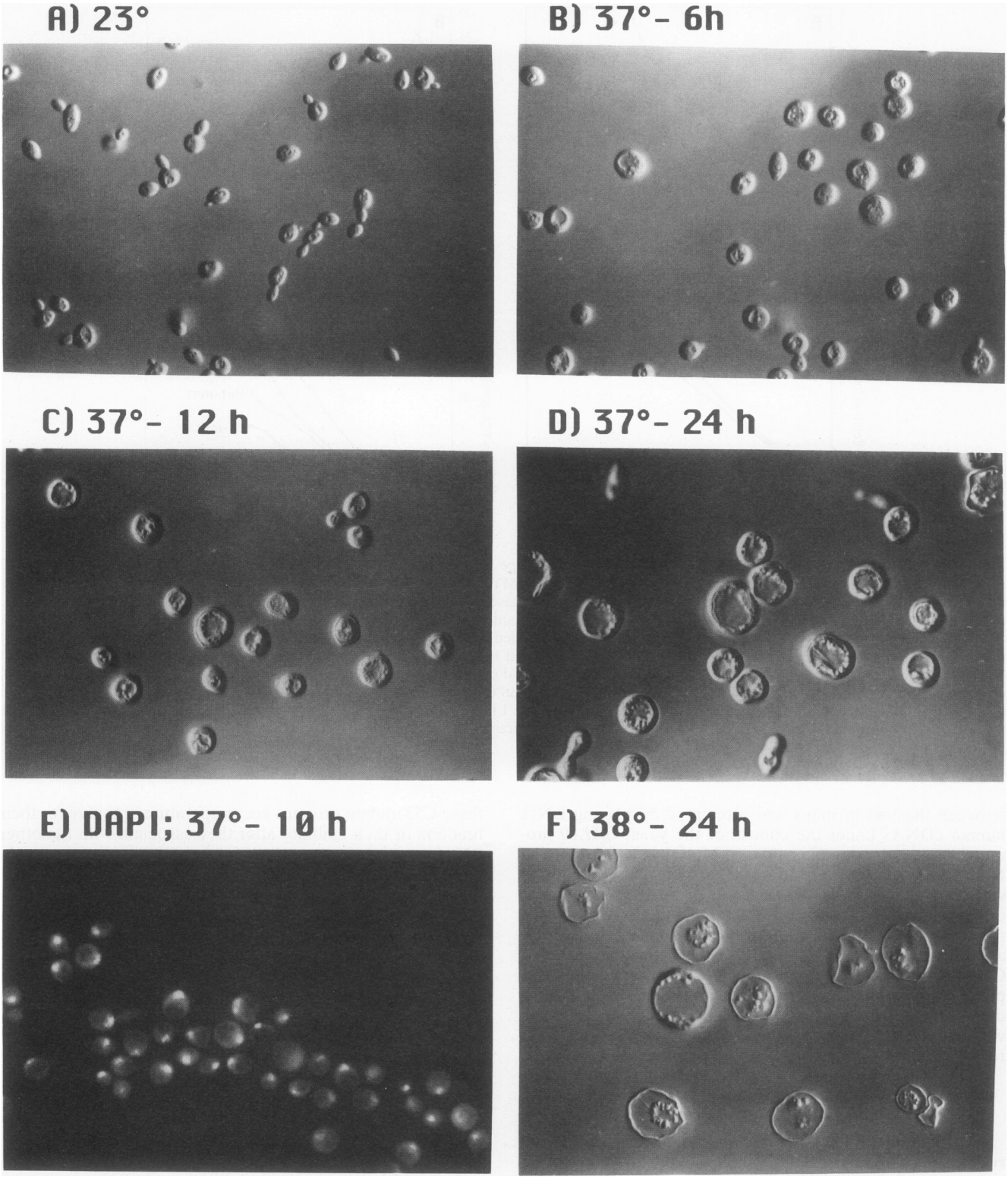


FIG. 7. Morphology of CMY397 *rpc53*::*HIS3-1* temperature-arrested cells. (A to D) Log-phase CMY397 cells in YPD at 23°C (A_{660} = 0.17; 2×10^6 cells per ml; 47% unbudded cells) were transferred to 37°C for the periods indicated in the figure. At each time point, an aliquot of cells was fixed in 3.7% formaldehyde and examined by differential interference contrast microscopy. (E) DAPI (4′,6′-diamidino-2-phenylindole) staining of CMY397 DNA after cells were incubated for 10 h at 37°C. Cells were fixed in 70% ethanol before being stained with 0.5 μg of DAPI per ml. The arrested cells exhibited a single nucleus. No punctate cytoplasmic staining is seen in these [*rho*^{0}] cells that lack mitochondrial DNA. (F) Terminal morphology of CMY397 cells after 24 h of incubation in YPD at 38°C. The mutant cells arrest their division and lyse more rapidly at 38°C than at 37°C. All the cells in this figure are shown at the same magnification (approximately ×880).

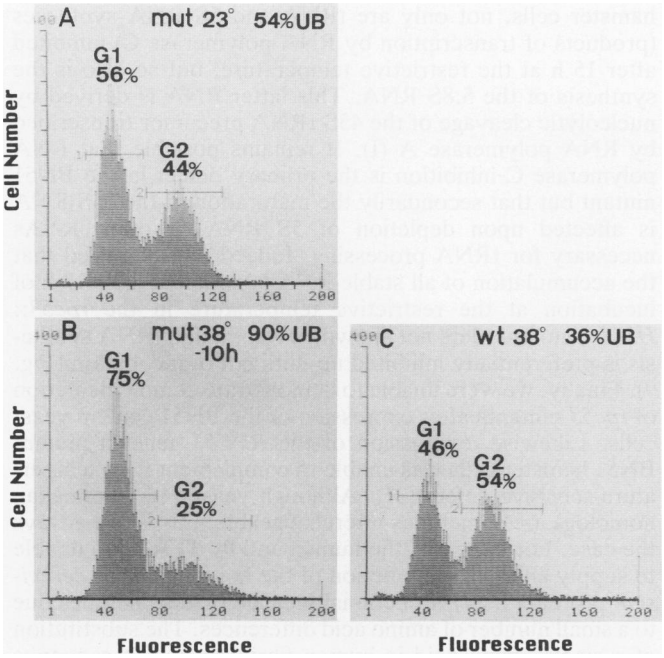


FIG. 8. Flow cytometry to quantify the DNA content of yeast cells. CMY397 *rpc53::HIS3-1* [*rho*^{0}] cells (mut in the figure) in YPD at 23°C (A) and after 10 h of incubation at 38°C (B) were fixed in 70% ethanol and stained with propidium iodide for FACS analysis. Also shown are CMY396 *rpc53::HIS3-1* [*rho*^{0}]/pEMBLYc32-*RPC53* control cells (wt in the figure) in log-phase growth at 38°C in YPD. (C) Plot of the number of cells (vertical axis) emitting a given arbitrary unit of fluorescence (horizontal axis) in proportion to the DNA content of each cell. The peaks labeled G_1 and G_2 correspond to peaks with a haploid or diploid content of DNA. S-phase cells are poorly resolved between the G_1 and G_2 peaks in this analysis, and no attempt was made to define this subpopulation of cells. It is nevertheless clear that CMY397 mutants arrest their cell division preferentially with a G_1 content of DNA after incubation at 38°C. No such accumulation of G_1 cells is seen for the CMY396 *RPC53*$^+$ cells grown at 38°C. We consistently observed a slightly lower percentage of G_1-phase cells, as determined by FACS analysis, than of unbudded (UB) cells, as seen by light microscopy, for CMY397 temperature-arrested mutants. This may mean that a fraction of the unbudded cells arrest with an S/G_2 content of DNA, or it may be due to experimental error in the classification of budded versus unbudded or G_1 versus G_2 cells.

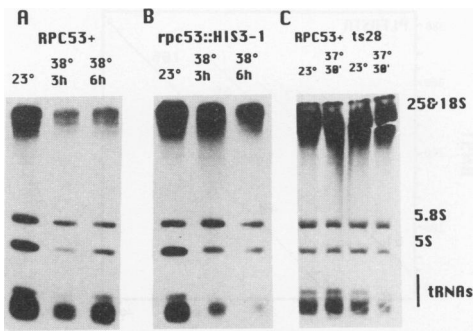
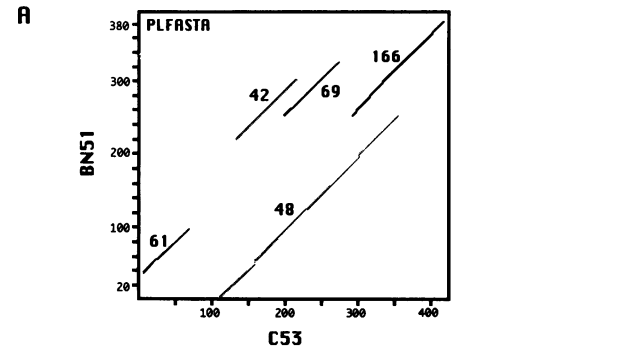


FIG. 9. Synthesis of small RNA molecules in *rpc53* mutants. CMY396 *rpc53::HIS3-1*/pEMBLYc-*RPC53* (A) and CMY397 *rpc53::HIS3-1* (B) cells were grown in synthetic complete medium and labeled with [^{3}H]uracil for 60 min at 23 or 38°C after 3 or 6 h of incubation. Small RNAs were extracted, and 20 μg of RNA from each sample was analyzed by electrophoresis in a 6% polyacrylamide gel and then by fluorography. (C) Small RNA synthesis was also examined in CMY *rpc53-ts*28 point mutants by [^{3}H]uracil labeling for 60 min at 23°C and after 30 min of incubation at 37°C. In all instances, a specific inhibition of tRNA accumulation was observed in the *rpc53* mutants at the restrictive temperature.

thesis under conditions of RNA polymerase C limitation. Transcription of 5S RNA genes requires the protein TFIIIA as well as the factors TFIIIB and TFIIIC, whereas tRNA gene transcription requires TFIIIB and TFIIIC but not TFIIIA (11, 13). Perhaps RNA polymerase C has a higher affinity and thermostability when associated with TFIIIA, -B, and -C compared with TFIIIB and -C alone. Under this hypothesis, it might be expected that with purified components, a greater affinity and thermoresistance would be found for 5S RNA compared with tRNA gene transcription in vitro. A second possibility is suggested by the exceptional localization of the 5S RNA genes to the nucleolus in *S. cerevisiae* (43). RNA polymerase C may have a higher affinity for 5S RNA genes in the nucleolus and be more stable there than when it is in the nucleoplasm. However, transfer of a marked 5S RNA gene to an autonomously replicating plasmid in yeast cells showed that the plasmid-borne 5S RNA genes (presumably nucleoplasmic) were preferentially transcribed relative to the nucleolar 5S RNA genes (59). It would nevertheless be interesting to determine whether this preference remained under conditions of RNA polymerase C limitation. This model also predicts that tRNA and 5S RNA synthesis would be equally affected by RNA polymerase C mutations in eukaryotes in which the 5S RNA genes are not localized to the nucleolus.

Preferential G_1 arrest of *rpc53* mutants. An unexpected phenotype of the *rpc53* mutants was their rapid and preferential arrest in the G_1 phase of the cell cycle as large, round, unbudded cells (Fig. 6 to 8). The arrested cells maintain high levels of protein synthesis and viability at the restrictive temperature but do not exhibit the shmoo morphology typical of *cdc28* mutants arrested in the G_1 phase at Start (18). Rather, their phenotype more closely resembles certain translational machinery mutants (*prt1/cdc63*; a translation initiation factor [17], *mes1*; methionyl-tRNA synthetase [57, 58]) in which a G_1 arrest is associated with only a partial inhibition of protein synthesis. It is believed, without having been demonstrated, that a partial inhibition of protein synthesis may lead to a specific arrest in G_1 by preferentially affecting the accumulation of unstable polypeptides (such as the G_1 cyclins) whose accumulation is necessary for the passage of Start in G_1 (44). Similarly, the G_1 arrest of the *rpc53* mutants might be the result of a partial inhibition of translation as a secondary consequence to the inhibition of stable RNA transcription by RNA polymerase C. An alternative hypothesis is that the C53 subunit is required for the transcription by RNA polymerase C of a nonabundant RNA species that is required for the passage of the G_1 phase. Based on drug inhibition studies, it was previously proposed that the transcription of some RNA species was necessary for the performance of Start in yeast cells (27, 60). The U6 small nuclear RNA and the nuclear RNase P RNA (*RPR1*) genes are the only other genes apart from the tRNA and 5S genes that are presently known to be transcribed by RNA polymerase C in *S. cerevisiae* (31, 36). The transcription of these genes is inhibited in the *rpc160-41* mutant (31, 36). Interestingly, a reexamination of the *rpc160-41* mutant has shown that it accumulates as large, round, unbudded cells at 37°C as do the *rpc53* mutants (55). A particular tRNA, the



B

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25.0% identity in 136 aa overlap; init:  102, opt:  166

          300       310       320       330       340       350
C53:  LKKMN-NKPERFMVFQLPTRLPAFER-PAVKEEKEDMETQASDP--SKKKKNIKKKDTKD
      :.... .: : .. .:::. ::.  . ...:  :.........  :..:. . :...
BN51:LRELSLTKEEELLFLQLPDTLPGQPPTQDIKPIKTEVQGEDGQVVLIKQEKDREAKLAEN
          260       270       280       290       300       310

          360       370       380       390       400
ALSTRELA-GKVGSIRVHKSGKLSVKIGNVVMDIGKGAETTFLHDVIALSIADD-ASSAE
: . .:. X.:.:. ..:::.. . .:.:.:.:...:. .:.........X. ... .
ACTLADLTEGQVGKLLIRKSGRVQLLLGKVTLDVTMGTACSFLQELVSVGLGDSRTGEMT
          320       330       340       350       360       370

410       420
LLGRVDGKIVVTPQI
.::.:. :.: .:..
VLGHVKHKLVCSPDFE
      380
```

FIG. 10. Sequence similarity between the yeast C53 and the human BN51 proteins. (A) PLFASTA analysis showing regions of similarity between the two proteins. The numbers next to each diagonal line indicate the optimized FASTA score for the different local regions of similarity. (B) Shown is the FASTA alignment (ktup = 1) of the carboxy-terminal regions of the C53 and BN51 proteins. Evolutionarily conservative amino acid substitutions (PAM250 scores of zero or greater) are indicated with a single dot, in contrast to the double dots for identical amino acids. The Xs indicate the boundaries of the region with the highest local similarity between the C53 and BN51 proteins. These analyses were performed with the FASTA program package (40, 41).

U6 or RPR1 RNAs, or an as-yet-unidentified RNA species transcribed by the C enzyme may be preferentially required in the G_1 phase.

Homology between the yeast C53 and mammalian BN51 proteins. A computer search of the protein sequence data banks with the C53 sequence revealed a significant degree of sequence similarity with only one protein, the human BN51 protein (21, 22). These proteins have 25% amino acid identity and 78% similarity (allowing as conservative amino acid substitutions a PAM250 [40] score of zero or greater) over the 136 carboxy-terminal amino acids of the two proteins (Fig. 10). Several points argue that the sequence similarities between the two proteins represent a functional homology. The two proteins are of similar size and hydrophilicity, and both are localized in the nucleus. The carboxy-terminal region of the two proteins has the highest level of sequence similarity, and this region also corresponds to the functionally essential portion of the C53 subunit (5). The BN51 protein was originally identified by the ability of its cDNA to complement the temperature-sensitive G_1-phase arrest of a BHK (baby hamster kidney) cell mutant. Similarly, *rpc53* mutations in yeast cells lead to a preferential G_1 arrest. Finally, tRNA synthesis is thermolabile both in vivo and in vitro in both temperature-sensitive *rpc53* (Fig. 9) (5) and BN51 cell mutants (21). However, it is not yet possible to conclude that the BN51 protein is the mammalian homolog of the C53 subunit. In temperature-sensitive BN51 mutant hamster cells, not only are tRNA and 5S RNA syntheses (products of transcription by RNA polymerase C) inhibited after 15 h at the restrictive temperature, but so also is the synthesis of the 5.8S RNA. This latter RNA is derived by nucleolytic cleavage of the 45S rRNA precursor transcribed by RNA polymerase A (I). It remains possible that RNA polymerase C inhibition is the primary defect in the BN51 mutant but that secondarily the maturation of the 35S RNA is affected upon depletion of 5S RNA or other RNAs necessary for rRNA processing. Indeed, we observed that the accumulation of all stable RNA is inhibited after 12 h of incubation at the restrictive temperature in the *rpc53*::*HIS3-1* mutant (data not shown), even though tRNA synthesis is preferentially inhibited up until 6 h of incubation (Fig. 9). Finally, we were unable to demonstrate complementation of *rpc53* mutants after expression of the BN51 gene in yeast cells. Likewise, expression of the *RPC53* gene in mutant BN51 hamster cells was unable to complement their temperature-sensitive defect (21). Although yeast and mammalian homologs are sometimes interchangeable, this is not always the case. For example, the human and fly TFIID are unable to supply an essential function of the homologous *S. cerevisiae* protein (7, 14). Functional incapacity is sometimes due to a small number of amino acid differences. The substitution of a single amino acid in human fibrillarin to the sequence that exists in yeast fibrillarin is sufficient to correct a temperature-sensitive defect in the ability of the human protein to complement the yeast mutant (25). Polypeptides of approximately the molecular weight determined for the BN51 protein were found to be associated with highly purified preparations of RNA polymerase C from mouse plasmacytoma (51) and human KB (23) cells. It remains to be seen whether any of these correspond to the BN51 protein.

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